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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) We are entering a new era of medicine where genetic markers are going to be used to make clinical management decisions. My long term career goal is to further our understanding of the genetic alterations which characterize human breast cancer in a way that will eventually lead to early diagnosis, more effective treatment or prevention of the disease. The promise of research into breast cancer genetics is that it will provide us with new insights into the etiology of breast cancer that can be translated to strategies for early diagnosis and treatment for the larger population of women who develop breast cancer without having a genetic predisposition.				
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Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4-6
Key Research Accomplishments.....	6
Reportable Outcomes.....	6-7
Conclusions.....	7
References.....	7-8
Appendices.....	NA

INTRODUCTION

As a physician-scientist, I have had extensive training in clinical oncology and in molecular biology and genetics; I am ideally positioned to bridge the gap between the two. The academic award has represented an outstanding opportunity for me to critically appraise the emerging role of genetics in clinical breast cancer care and forge new avenues of research. Toward this goal, I plan to accomplish the following during the period of my academic award.

1) perform a thorough review of the cytogenetic and molecular genetics literature to identify potential chromosomal regions that may harbor genes whose abnormal function is critically involved in the development of breast cancer.

2) develop a robust panel of markers that can be used for clinical correlative studies of hereditary breast cancers.

3) develop a tissue repository composed of biological specimens from 500 patients with inherited breast cancer (e.g fresh frozen tumor specimens, or paraffin embedded tumor specimens and normal blood lymphocytes, DNA and sera whenever possible).

Using these unique resources, my future studies will characterize the molecular pathways that allow a normal breast cell to become cancerous in individuals who are genetically predisposed. I will also develop longitudinal follow up studies to correlate clinical outcomes with molecular characterization and epidemiologic risk factors. These studies will no doubt lead to an improved understanding of the biology of breast cancer which will ultimately translate into more effective therapies.

Task I

perform a thorough review of the cytogenetic and molecular genetics literature to identify potential chromosomal regions that may harbor genes whose abnormal function is critically involved in the development of breast cancer.

This year we published two reviews on the genetics of breast cancer. In the next year, we are completing two manuscripts that will focus on the chromosomal abnormalities and genetic alterations in breast cancer.

Publications

Olopade OI, Pichert G. Cancer genetics in oncology practice. *Ann Oncol* 2001 Jul;12(7):895-908

White, M, Bradbury, A, Olopade OI. Breast and Ovarian Cancer Risk Assessment and Risk Reduction: Strategies for the Primary Care Physician. In Press *Women's Health*

Task II

develop a robust panel of markers that can be used for clinical correlative studies of hereditary breast cancers.

We have developed several probes for fluorescent in situ hybridization and have begun to apply these probes to a panel of breast tumors in our tumor bank.

a) Dissection of Cooperating Oncogenes involved in *BRCA1* tumor progression

To examine whether amplification of *HER-2/neu* contributes to the aggressive biology of *BRCA1*-associated tumors, we performed fluorescence *in situ* hybridization (FISH) on formalin-fixed paraffin-embedded breast tumor tissue sections from 53 *BRCA1* mutation carriers and 41 randomly selected age-matched sporadic breast cancer cases. Although *BRCA1*-associated and sporadic tumors were equally likely (19% versus 22%) to exhibit *HER-2/neu* amplification (defined as a ratio of *HER-2/neu* copies to chromosome 17 centromere (*CEP17*) copies ≥ 2), 6 (15%) of the sporadic tumors were highly amplified (defined as a ratio ≥ 5) versus none of the *BRCA1*-associated tumors ($p = 0.048$). *HER-2* protein overexpression as measured by immunohistochemical analysis (IHC) was not observed among the *BRCA1*-associated cases ($p = 0.042$). Four out of 21 (19%) sporadic tumors exhibited strong membranous staining of *HER-2* (intensity level of 3+) as compared to 0/39 *BRCA1*-associated tumors. Our data suggest that a germ line mutation in the *BRCA1* tumor suppressor gene is associated with a significantly lower level of *HER-2/neu* amplification. Thus, it is possible that *BRCA1*-associated and *HER-2/neu*-highly amplified tumors progress through distinct molecular pathways and the aggressive pathologic features of *BRCA1*-associated tumors appear unrelated to amplification of the adjacent *HER-2/neu* oncogene (Grushko et al. 2002).

We further examined whether *MYC* amplification contributes to tumor progression in *BRCA1*-associated human breast cancer, and analyzed tumors using a *MYC/CEP8* assay on formalin-fixed paraffin-embedded tumor tissues from 28 women with known deleterious germ line *BRCA1* mutations and 49 sporadic cases, including 18 with hypermethylation of the *BRCA1* gene promoter. We observed a *MYC/CEP8* amplification ratio ≥ 2 in 17 of 28 (61%) *BRCA1*-mutated and in 13 of 49 (27%) sporadic tumors ($P = 0.009$). Of the 13 sporadic cases with *MYC* amplification, 7 (54%) were *BRCA1*-methylated. In total, *MYC* amplification was found in the majority of tumors with inactivated *BRCA1* (24/46, 52% versus 6/31, 19%; $p = 0.01$). We concluded that *MYC* oncogene amplification is associated with multi-step tumor progression in both *BRCA1*-associated hereditary and methylated sporadic tumors, which suggests that *BRCA1* promoter methylation may be an early step in the development of some sporadic cancers. We hypothesize that *BRCA1* may function as a tumor suppressor gene in part by regulating *MYC* oncogenic activity.

b) . Hypermethylation of *BRCA1* and ER promoter

We assessed *BRCA1* and estrogen receptor (ER) promoter methylation in 5 breast cancer cell lines and 132 primary breast tissues by Methylation-Specific (M-PCR). *BRCA1* and ER expression were determined in breast tumor cell lines and primary tissues by RT-PCR. In addition, we performed FISH using *BRCA1* and *CEP17* probes on both sporadic and *BRCA1*-associated hereditary breast cancer. We observed *BRCA1* methylation in the UACC-3199 positive control cell line and in 39 of 132 sporadic (29.5%) tumors. *BRCA1* methylation was correlated with chromosome 17 aneusomy and down-regulation or complete absence of the transcript. *BRCA1* methylation correlated inversely with age of onset: 40% of tumors from cases under 55 years old were methylated vs. 25% of cases over 55 years old (Table 4). The methylated cases were equally distributed among all histological types and there was no difference in the proportion of African American women (27.5%) vs. non-Hispanic White women with methylated tumors (28.6%). The majority of *BRCA1*-methylated cases (79%) were ER (-) and/or ER methylated. *MYC* and *HER2/neu* amplification in methylated tumors were intermediate in values between hereditary *BRCA1*-associated and sporadic unmethylated tumors, suggesting that *BRCA1* methylation might be incomplete in some tumors. These results suggest that silencing of the *BRCA1* gene by methylation occurs in a significant proportion of sporadic breast cancers and may be an early event during tumor progression (Wei et al. in preparation). There may be a slight difference in the proportion of black women with methylated tumors.

Publications

Grushko TA, Blackwood MA, Schumm PL, Hagos FG, Adeyanju MO, Feldman MD, Sanders MO, Weber BL, Olopade OI. Molecular-cytogenetic analysis of HER-2/neu gene in BRCA1-associated breast cancers. *Cancer Res.* 2002 Mar 1;62(5):1481-8.

Min-Jie Wei¹, Tatyana Grushko¹, Soma Das², James Dignam³, Fitsum Hagos¹, Lise Sveen¹, James Fackenthal¹ and Olufunmilayo I Olopade¹. Methylation of the *BRCA1* Promoter in Sporadic Breast Cancer Related to *BRCA1* Copy Number and Pathologic Features. Manuscript in preparation

Tatyana A. Grushko,, James J. Dignam, , Soma Das, Anne Blackwood, Charles M. Perou, , April J. Adams, , Fitsum G. Hagos, Lise Sveen , Karin K. Ridderstråle, Kristin Anderson, Barbara L. Weber Olufunmilayo I. Olopade, M.D. *MYC* is amplified in *BRCA1*-associated breast cancers. Manuscript in preparation

Task III

develop a tissue repository composed of biological specimens from 500 patients with familial or hereditary breast cancer (e.g fresh frozen tumor specimens, or paraffin embedded tumor specimens and normal blood lymphocytes, DNA and sera whenever possible).

We have developed a clinical protocol for the tumor bank. The protocol has not yet been approved by the DOD Human Subjects Review Panel. Hence we have not enrolled any patients specifically to this study. However, we have identified collaborators and other sources of tumor materials that will be ready and available for recruitment once our study is approved. Our protocol is still awaiting approval by the DOD.

KEY RESEARCH ACCOMPLISHMENTS:

We are defining important pathways in BRCA1 tumor progression.

REPORTABLE OUTCOMES:

Academic Productivity in 2002.

1. Olopade OI, Fackenthal JD, Dunston G, Tainsky MA, Collins F, Whitfield-Broome C. Breast cancer genetics in African Americans. *Cancer.* 2003 Jan 1;97(1 Suppl):236-45. PMID: 12491487

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4: Antman K, Abraido-Lanza AF, Blum D, Brownfield E, Cicatelli B, Debor MD, Emmons K, Fitzgibbon M, Gapstur SM, Gradishar W, Hiatt RA, Hubbell FA, Joe AK, Klassen AC, Lee NC, Linden HM, McMullin J, Mishra SI, Neuhaus C, Olopade FI, Walas K. Reducing disparities in breast cancer

survival: a Columbia University and Avon Breast Cancer Research and Care Network Symposium. Breast Cancer Res Treat. 2002 Oct;75(3):269-80.
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9: Grushko TA, Blackwood MA, Schumm PL, Hagos FG, Adeyanju MO, Feldman MD, Sanders MO, Weber BL, Olopade OI. Molecular-cytogenetic analysis of HER-2/neu gene in BRCA1-associated breast cancers. Cancer Res. 2002 Mar 1;62(5):1481-8.

CONCLUSIONS:

The observed similarities between *BRCA1*-mutated and *BRCA1*-methylated tumors led us to propose a tumor progression model in which early loss of *BRCA1* causes defects in chromosome structure, cell division, and viability, so that a *BRCA1*-deficient cell must acquire additional alterations that overcome these problems and presumably force tumor evolution down a limited set of pathways. Our FISH results are consistent with data from DNA microarray studies that suggest that breast cancers arising in the setting of germ line *BRCA1* mutations have unique gene expression profiles, and sporadic tumors with methylated *BRCA1* may be misclassified with the *BRCA1*-mutation-positive group. A review of the set of genes published by Hedenfalk et al. (Hedenfalk et al. 2001) and in the recent paper by van 't Veer et al. (van 't Veer et al. 2002) demonstrated that *MYC* on 8q was overexpressed in *BRCA1* mutation carriers. In addition, the *BRCA1* mutant tumors we have studied appear to have a profile that is most consistent with the basal-like subtype suggested by Perou et al. (Perou et al. 2000), based upon the following observations. First, both (meaning sporadic basal-like tumors and *BRCA1* mutant tumors) tend to be high grade, ER/PR negative and *HER2/neu*-negative, and both show the high expression and/or amplification of *MYC*. In fact, *MYC* emerged as one of the most relevant genes that defined the basal-like group and was expressed more than 2-4 fold above background in the majority of cases (Perou, unpublished results). Moreover, we have previously shown that *BRCA1*-mutated tumors express specific basal cytokeratins in a manner suggestive of an ER-negative basal-like epithelial cell of origin (Olopade and Grushko 2001) and are never associated with high levels of *HER-2/neu* amplification (Grushko et al. 2002). Therefore, it is reasonable to suggest that *BRCA1*-mutated tumors are mostly basal-like (ER-, *HER2*-) and that *MYC* amplification/overexpression further defines *BRCA1*-deficient tumor cells.

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Molecular portraits of human breast tumours.

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APPENDICES:

N/A